Kidney International, Vol. 46 (1994), pp. 807–813

Increased cardiovascular risk factors and features of endothelial activation and dysfunction in dialyzed uremic patients

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Increased cardiovascular risk factors and features of endothelial activation and dysfunction in dialyzed uremic patients. Due to the incidence of symptomatic atherosclerosis in uremic patients, hemostasis-derived cardiovascular risk factors, basal plasma concentrations of some endothelial-derived glycoproteins and desmopressin-induced variations of endothelial-derived proteins were studied in 22 uremic patients on prolonged maintenance hemodialysis with no cardiovascular antecedent. Compared to control subjects, patients had increased predialysis hemostasis-related cardiovascular risk factors: high fibrinogen, proconvertin, and type 1 plasminogen activator inhibitor plasma concentrations; low albumin values; generally low antithrombin III values but sometimes high. They had high predialysis plasma concentrations of endothelium-derived glycoproteins: von Willebrand factor, tissue-type plasminogen activator and urokinase-type plasminogen activator, which are secreted by endothelial cells, but also soluble thrombomodulin, a marker of endothelial cell injury. The desmopressin-induced release of tissue-type plasminogen activator and of von Willebrand factor were lower than in controls. High fibrinogen, type 1 plasminogen activator inhibitor and low albumin plasma concentrations may be linked to repeated acute phase reactions associated with hemodialysis. Data concerning endothelium-related proteins are concordant with the co-existence of a chronic *in vivo* endothelial activation and endothelial injury in uremia. This could be linked to the initiation and progression of atherosclerosis.

Bleeding has been recognized as a complication of uremia as early as 1907 [1]. Maintenance hemodialysis has reduced the incidence of hemorrhage but is paradoxically associated to accelerated atherosclerosis [2] and to increased atherogenesis-related cardiovascular mortality [3, 4]. Endothelial dysfunction is classically thought to be central to atherogenesis [5] and an impaired venous-occlusion induced release of two endothelium-derived glycoproteins, namely von Willebrand factor (vWF) and tissue-type plasminogen activator (t-PA) has been described in hemodialysis patients though responses in undialyzed patients were normal [6]. Endothelial dysfunction characterized by decreased t-PA related antigen (t-PA Ag) release, raised basal plasma vWF related antigen (vWF Ag) concentration and transcapillary albumin escape [7] has been associated with early diabetic nephropathy. Short lasting infusions of deamino-8-D-arginine vasopressin (dDAVP; desmopressin) can be used for temporary correction of the bleeding time in uremia [8] and induce the release of some of the substances present in the endothelial cells: vWF, t-PA [reviewed 9] and urokinase-type plasminogen activator (u-PA) [10].

Prospective studies have already established a strong relationship between the subsequent incidence of cardiovascular diseases and the plasma levels of the following so-called cardiovascular risk factors: fibrinogen (Fg) [11–13], coagulation factor VII (proconvertin: FVII) [12] and type 1 plasminogen activator inhibitor (PAI-1) [14, 15].

Because certain laboratory abnormalities were observed to occur in the patients with chronic renal failure [6, 16–20], a prospective study was designed to examine hemostasis-derived cardiovascular risk factors, endothelium-derived plasma proteins and desmopressin-induced variations of some endothelium-derived specific factors in these patients and compare them to similar measurements in normal subjects.

Methods

Patients

Records of 43 patients started on maintenance hemodialysis in the Department of Nephrology of Nîmes Hospital were examined. Patients having a past history of angina pectoris, myocardial infarction, transient ischemic attack or complete stroke, acute or symptomatic chronic peripheral arterial ischemia, diabetes, vasculitis or systemic lupus erythematosus were excluded. All remaining patients who gave their informed consent to participate to this study were included. All had normal electrocardiographic patterns.

Twenty-two uremic patients (13 males and 9 females, median age 59, range 18 to 77) entered the study. Renal failure had resulted from polycystic disease in five and glomerulonephritis in seventeen. All patients received the same treatment: 9 to 10.5 hours a week on hollow fiber^R dialyzers (Bellco, Mirandola, Italy; 1.3 m^2 area) with cuprophan membranes (8 μ). The blood flow was kept at 300 ml/min. The dialysate, used single pass, had the following composition (in mmol/liter): Na 138, K 1.5, Ca 1.75, Mg 0.75, Cl 107, HCO_3 34. The diet was low in salt (no added salt) with a mean protein intake $>$ 1 g/kg body weight. Vascular connections with the hemodialysis device had been done by way of a previously performed arteriovenous fistula. The median duration of dialysis treatment was 46 months (range 1 to 183 months). None of the patients had any evidence of illness other than uremia. The pre-dialysis mean arterial pressure (MAP) was

Received for publication May 10, 1993
and in revised form April 13, 1994
Accepted for publication April 14, 1994

calculated [MAP = diastolic + (systolic − diastolic)/3] for each patient and the month before the biological investigation (from average value of all sessions of the month).

Patients with at least one vascular thrill (except for arteriovenous fistula) or at least one non-pulsatile peripheral artery were considered as positive for peripheral vascular disease (PVD). Patients who had suffered from arteriovenous fistula thrombosis were classified as "fistula thrombotic patients" (FTP). None of the patients had suffered from deep vein thrombosis.

A control group of 22 healthy volunteers (aged 52, range 22 to 65, 13 men and 9 women) was recruited among the medical staff and their friends at the same time as their matched uremic patient.

Design of the study

DDAVP (desmopressin; MINIRIN^R, Ferring AB pharmaceuticals, Malmo, Sweden) was given intravenously (0.3 μg/kg body wt). The total dDAVP dose was diluted in 100 ml saline solution, and infused over a 30 minute period, between 9.00 and 11.00 a.m.

Uremic patients had been first dialyzed 48 hours before dDAVP infusion. Desmopressin was infused using the venous network of the forearm with no arteriovenous fistula.

A venous blood sample was taken from the antecubital vein of the forearm planned for infusion five minutes before the beginning of dDAVP administration. An arterial blood sample was taken five minutes after the end of infusion through an arteriovenous fistula puncture (uremic patients) or a radial artery puncture (control patients; forearm opposite to dDAVP infusion).

Collection of blood

All vessel punctures were done using a 19 gauge needle and no tourniquet. For hemostasis related assays, blood was collected in silicone tubes containing 1 volume per 10 of the "C.T.A.D." anticoagulant-antiplatelet solution (3.8% trisodium citrate, theophylline, adenosine, dipyridamol; Diatube^R H, Diagnostica Stago, Asnières, France) [21] after systematic rejection of the first 2 ml of blood and were centrifuged for 20 minutes at 2,500 g, at room temperature. Tubes for fibrinolysis related assays were immediately immersed in melting ice and centrifuged at 4°C. Plasma samples were stored at −85°C until assayed.

Laboratory methods

Non-hemostasis related assays. Due to desmopressin-induced plasma volume variations, post-dDAVP values were corrected by using a factor F defined as [100 minus packed cell volume (PCV) after infusion]/[100 minus PCV before infusion].

Serum albumin concentrations were measured by laser nephelometry (BNA, Behringwerke AG, Marburg, Germany).

Hemostasis related assays. All clotting assays were performed on ST888 analyzer (Diagnostica Stago). Chromogenic assays were performed on ST888 or on a microplate reader (Titertek Twinreader^R, Flow Laboratories SA, Putaux, France). Enzyme linked immunosorbent assays (ELISAs) were read using the Twinreader^R.

Clottable fibrinogen (Fg) was measured using Clauss' method [22] (Fibri Prest^R, Diagnostica Stago).

Factor VII activity (FVII activity) was determined by a synthetic chromogenic method according to Seligsohn, Osterud and Rapaport [23] (Stachrom^R VII, Diagnostica Stago).

Chromogenic assays were used for type 1 plasminogen activator inhibitor activity (PAI-1 activity) [Spectrolyse™ (pL) PAI, Biopool, Umea, Sweden] [24] and for antithrombin III activity (AT III; Stachrom^R ATIII, Diagnostica Stago) [25].

Factor VII related antigen (FVII Ag) and von Willebrand factor related antigen (vWF Ag) were assayed by ELISAs (Asserachrom^R VII and Asserachrom^R vWF, Diagnostica Stago) [26, 27].

Type 1 plasminogen activator inhibitor-related antigen (PAI-1 Ag) was quantified using a monoclonal ELISA (Tintelize™ PAI-1, Biopool AB) [28]. This assay has been previously shown to be relatively insensitive to changes in complexation of PAI-1 with single-chain tissue type plasminogen activator and with urokinase-type plasminogen activator, and its low reactivity to latent PAI-1 renders this method relatively insensitive to contamination of plasma with platelet release products [28].

Type 2 plasminogen activator inhibitor-related antigen (PAI-2 Ag) plasma concentrations were measured by a monoclonal ELISA (Tintelize™ PAI-2, BiopoolAB) in which the coated catching antibody is the one described by Astedt et al [29].

Tissue-type plasminogen activator related antigen (t-PA Ag) was assayed by an ELISA (Tintelize™ t-PA, Biopool), which uses the monoclonal antibody referred to as 1:3D3 in Stigbrand's characterization work [30]: this antibody recognizes with a high affinity a conformation dependent epitope on single-chain and two-chains t-PA, and binds to t-PA/PAI-1 complexes.

Urokinase-type plasminogen activator (u-PA Ag) was measured with an ELISA reagent kit (Tintelize™ u-PA, Biopool AB) which uses a previously described catching monoclonal antibody [31]. This antibody binds to single-chain urokinase-type plasminogen activator (scu-PA) and to high molecular weight two-chain urokinase type plasminogen activator (HMW/tcu-PA), but not to low molecular weight/tcu-PA.

Soluble thrombomodulin (TM) plasma levels were quantified by ELISA (Asserachrom^R Thrombomodulin, Diagnostica Stago) [32]. Thrombomodulin is a glycoprotein that is mainly present on the cell surface membranes of endothelium. Its soluble plasma fraction derives from the degradation of endothelial thrombomodulin by various proteases in the process of vascular endothelial cell destruction [33].

All these assays were calibrated using reference plasmas and purified standards given by the manufacturers. All assays were performed in triplicate and we used the obtained median values. For each test, within-assay variations ($N = 10$) and between-assay variations ($N = 10$) were determined using 15 different plasmas (5 low values, 5 normal values, 5 high values). In a given test, we used the worse value of the obtained within-assay and between-assay variations as the final coefficient of variation. Desmopressin induced variations of values (that is, "delta" parameter: value after infusion minus value before infusion) were calculated. In this case, for a given biological test, only delta values greater or lower than two final coefficient of variation values were considered as individual significant variations. Other values were considered nonsignificant and used as null values.

Statistical analysis

Results are given as median and range values.

Comparisons between groups of data were performed using non-parametric tests: the Mann-Whitney rank sum U test (non-paired data) and the Wilcoxon's signed-rank T test (paired data). $P > 0.05$ was considered not significant. In order to limit the

Table 1. Values of predialysis biological assays obtained in the control group (C) and in the patient group (P)

Parameter	Group	Median value	Minimum value	Maximum value	*P* Mann-Whitney
Albumin	C	47.9	43.2	58.8	
g/liter	P	38.2	29.7	47.8	10^{-5}
Packed cell	C	41.6	35.2	45.3	
volume %	P	25.6	21.0	33.2	$1.4\ 10^{-8}$
Fibrinogen	C	2.50	1.72	3.62	
g/liter	P	3.80	2.25	6.75	$4\ 10^{-7}$
F VII activity	C	94	70	113	
%	P	116	103	133	$1.6\ 10^{-6}$
F VII Ag	C	96.0	71.5	116	
%	P	129	96	186	$3\ 10^{-6}$
PAI-1 activity	C	5.3	1.6	17.0	
U/ml	P	27.5	2.1	37.7	$6\ 10^{-6}$
PAI-1 Ag	C	10.7	2.4	21.4	
ng/ml	P	13.8	2.8	46.5	$2\ 10^{-2}$
vWF Ag	C	100.5	64.5	156.0	
%	P	199.0	94.5	344.0	$6\ 10^{-6}$
t-PA Ag	C	4.2	1.3	9.1	
ng/ml	P	6.5	3.6	21.5	$2\ 10^{-3}$
u-PA Ag	C	0.29	0.19	0.53	
ng/ml	P	0.59	0.29	1.40	10^{-6}
PAI-2 Ag	C	2.4	1.0	4.2	
ng/ml	P	2.2	0.0	14.9	0.54 NS
AT III activity	C	94.5	86.0	103.0	
%	P	83.5	65.0	118.0	10^{-2}
Soluble TM	C	29.5	16.5	51.0	
ng/ml	P	198.0	98.0	444.0	10^{-8}

Abbreviations are: F VII activity, proconvertin activity; FVII Ag, proconvertin related antigen; PAI-1 activity, type 1 plasminogen activator inhibitor activity; PAI-1 Ag, type 1 plasminogen activator inhibitor-related antigen; vWF Ag, von Willebrand factor-related antigen; t-PA Ag, tissue-type plasminogen activator-related antigen; u-PA Ag, urokinase-type plasminogen activator-related antigen; AT III activity, antithrombin III activity; soluble TM, plasma soluble thrombomodulin-related antigen.

generation of Type II errors, only statistical comparisons which type I error risk (false positive risk) had been calculated to be below 0.05 and which type II error risk (false negative risk) corresponding to a 5% type I error risk was also below 5% were considered significant.

Limited correlations between data were performed using Spearman's coefficient of rank correlation "*r*'".

Results

The median mean arterial pressure value found in our patients on regular hemodialysis was 100 mm Hg (range 76.8 mm Hg to 114.7 mm Hg). They had significantly lower packed cell volume (PCV) and serum albumin concentrations than controls (Table 1). On the other hand, uremic patients had higher plasma concentrations of the established cardiovascular risk factors: fibrinogen, FVII activity, FVII Ag, PAI-1 activity and PAI-1 Ag. The endothelium-derived proteins t-PA Ag, u-PA Ag, vWF Ag and soluble thrombomodulin (TM) were also higher in patients than in controls. Uremic patients had generally decreased AT III activities, but three had higher AT III than the maximum value found among members of the control group.

Desmopressin infusions performed in members of the control group and in patients induced a significant decrease of albumin concentrations (values are given in Table 2 and Fig. 1. Wilcoxon paired t-test, $P = 1.3\ 10^{-5}$ and $9.8\ 10^{-3}$, respectively). DDAVP also induced an increase of t-PA Ag, vWF Ag and u-PA Ag

Table 2. Matrix of Spearman's rank correlations obtained between some predialysis haemostasis related parameters in patients

	PAI-1 Ag	vWF Ag	t-PA Ag	TM
PAI-1 Ag		0.425	0.838	0.733
P		0.048	0.0001	0.0008
vWF Ag			0.496	0.428
P			0.023	0.049
t-PA Ag				0.894
P				0.0000
TM				
P				

Abbreviations are: PAI-1 Ag, type 1 plasminogen activator inhibitor-related antigen; vWF Ag, von Willebrand factor-related antigen; t-PA Ag, tissue-type plasminogen activator-related antigen; TM, plasma soluble thrombomodulin-related antigen.

(control group, $P = 7.6\ 10^{-6}$ for the 3 dDAVP induced variations; patients, respectively $P = 1.3\ 10^{-5}$, $P = 7.6\ 10^{-6}$ and $P = 1.0\ 10^{-4}$). Patients had dDAVP-induced lower releases of t-PA Ag and vWF Ag than those evidenced in controls (Mann-Whitney U test; Fig. 1). They also experienced a lower desmopressin induced decrease of serum albumin values.

In patients, none of the predialysis hemostasis parameters values were significantly correlated with the existence of a past history of arteriovenous fistula thrombosis (AVF) or with the presence of asymptomatic peripheral vascular disease (PVD). Peripheral vascular disease was only significantly correlated to the cumulative number of performed dialysis treatments ($r' = 0.560$, $P = 0.01$). PAI-1 Ag, t-PA Ag, vWF Ag and TM values are correlated in Table 3.

Studies undertaken in the control group between some of the desmopressin induced variations of biological parameters (Δ parameters: Δ albumin, Δ t-PA Ag, Δ u-PA Ag, Δ vWF Ag, Δ PAI-1 Ag) only showed a positive correlation between Δ t-PA Ag and Δ vWF Ag ($r' = 0.647, P = 0.003$) and a negative one between Δ albumin and Δ PAI-1 Ag ($r' = -0.647, P = 0.003$). In patients, arteriovenous fistula thrombotic antecedents and asymptomatic peripheral vascular disease clinical symptoms were not correlated to these dDAVP induced variations. The number of performed dialysis treatments was positively correlated to Δ albumin ($r' = 0.695, P = 0.002$) but negatively to Δ PAI-1 Ag ($r' = -0.702, P = 0.001$), Δ t-PA Ag ($r' = -0.628, P = 0.004$) and to Δ vWF Ag ($r' = -0.505, P = 0.021$). There was a weak correlation between Δ t-PA Ag and Δ vWF Ag, while Δ t-PA Ag, Δ PAI-l and Δ PAI-1 Ag were altogether highly correlated (Table 3). Serum albumin variations were negatively linked to PAI-1 Ag and t-PA Ag variations.

Discussion

Since there is accumulating evidence that cardiovascular complications represent the first cause of mortality in patients with chronic renal failure, we decided to perform studies of the so-called hemostasis-derived cardiovascular risk factors in patients who are on regular hemodialysis treatment. As endothelium is thought to be involved in the atherogenetic process, we also assayed basal plasma concentrations of some endothelium-derived glycoproteins. We finally used desmopressin infusions as a tool to study one of the endothelial cell properties: the release capacity of intracellular t-PA and vWF.

The studied hemostasis-derived cardiovascular risk factors were

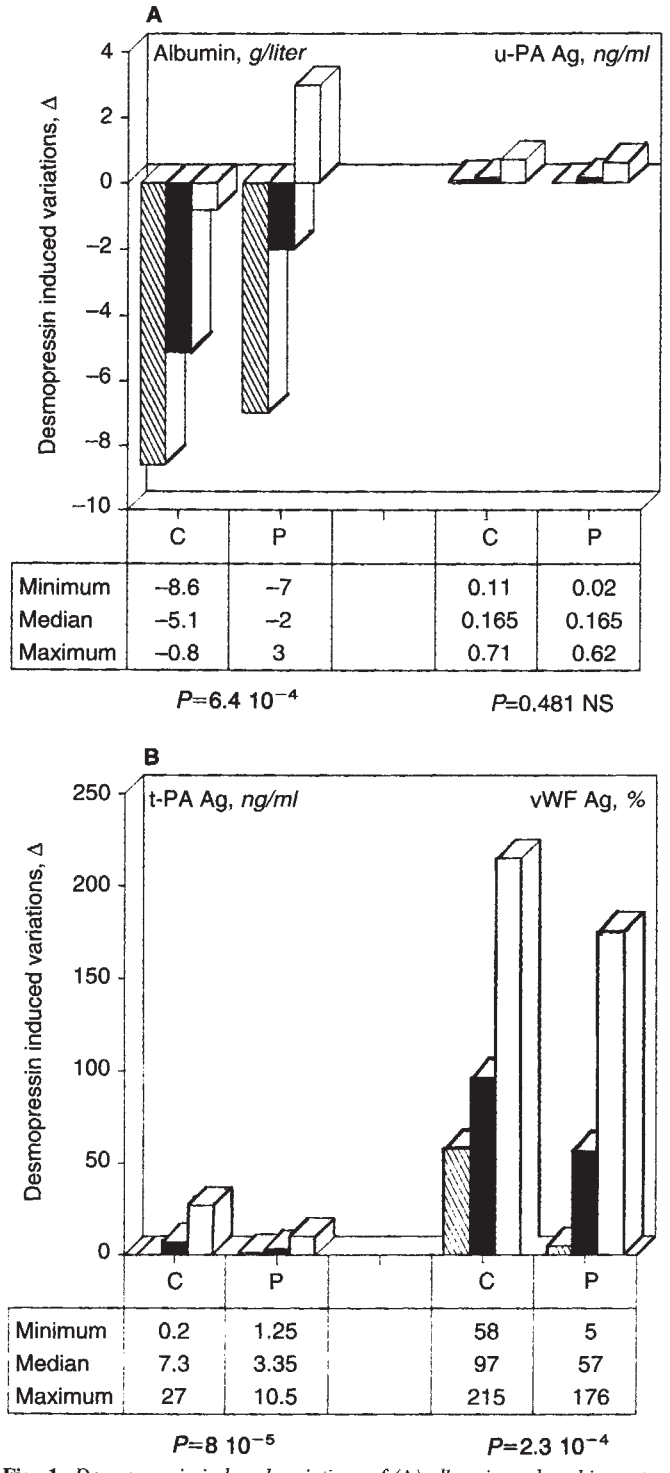

Fig. 1. *Desmopressin induced variations of* (**A**) *albumin and urokinase-type plasminogen activator-related antigen (u-PA Ag), and* (**B**) *tissue-type plasminogen activator antigen (t-PA Ag) and von Willebrand factor antigen (vWF Ag) obtained in the control group (C) and in patients (P).* Variations are given using the delta parameter (value after dDAVP infusion minus value before dDAVP infusion). Mann-Whitney's U test was used to perform statistical comparisons.

fibrinogen, factor VII (antigen and coagulant activity) and type 1 plasminogen activator inhibitor (antigen and activity). All these parameters were higher in patients than in controls. High values

Table 3. Matrix of Spearman's rank correlations obtained between some desmopressin induced variations (Δ parameter) of hemostasis related parameters in patients

	Δ Albumin	Δ PAI-1 Ag	Δ t-PA Ag	Δ vWF Ag	Δ u-PA Ag
Δ Albumin		−0.964	−0.856	−0.255	0.197
P		<0.0001	0.0001	0.243	0.367
Δ PAI-1 Ag			0.875	0.365	−0.148
P			0.0001	0.095	0.498
Δ t-PA Ag				0.488	0.001
P				0.025	0.998
Δ vWF Ag					−0.221
P					0.312
Δ u-PA Ag					
P					

Abbreviations are: PAI-1 Ag, type 1 plasminogen activator inhibitor-related antigen; vWF Ag, von Willebrand factor-related antigen; t-PA Ag, tissue-type plasminogen activator-related antigen; u-PA Ag, urokinase-type plasminogen activator-related antigen.

of these parameters are classically associated with a high risk of arterial thrombotic disease [11–15], and hyperfibrinogenemia or high proconvertin plasma concentrations have been consistently found in uremia [16, 17]. Predialysis antithrombin III plasma levels were lower in patients than in controls; this is concordant to previously published data [17]. Three out of the 22 patients had plasma ATIII concentrations higher than extreme values found in controls. Prospective studies in the Northwick Park Heart Study have led Meade et al to suggest that both low and high levels of ATIII may be associated with the risk of death from arterial disease [34]. Low predialysis serum albumin concentrations were also found in patients. Hemodilution due to water retention together with prolonged reduction of protein intake should contribute to hypoalbuminemia. However, Phillips, Shaper and Whincup have previously shown a general highly significant association between low serum albumin concentrations and mortality from cardiovascular diseases [35]. Thus, in patients on regular dialysis treatment, all studied plasma parameters where basal concentrations are known to be associated to arterial thrombotic events indicated a higher cardiovascular risk than in controls. The underlying mechanisms deserve to be investigated. Fibrinogen and PAI-1 are acute phase proteins which rise in response to several stimuli, including soluble cytokines like interleukin-1 (IL-1), tumor necrosis factor (TNF) and interleukin 6 (IL-6) [36–38]. These cytokines suppress household genes, and particularly the albumin gene [reviewed in 39]. Atherosclerosis is a chronic inflammatory disorder. Due to the simultaneous presence of IL-1 and TNF-α in the plasma of long-term hemodialysis patients, hemodialysis has been assimilated to a recurrent acute phase inflammatory response [40]. This may at least partly account for the observed fibrinogen, PAI-1 and albumin levels. High TNF-α concentrations have been recently demonstrated in human atherosclerotic arterial walls and could be actively involved in atherogenesis [41].

Patients also had higher predialysis plasma concentrations of endothelial-derived glycoproteins than controls, namely tissue-type plasminogen activator, von Willebrand factor, soluble thrombomodulin and urokinase-type plasminogen activator. The factor VIII complex has been extensively investigated in uremia and levels of vWF Ag are usually elevated [18, 19]. Von Willebrand

factor is synthesized predominantly by vascular endothelial cells and, to a lesser degree, by megakaryocytes [42, 43]. The administration of dDAVP to normal subjects has allowed the calculation of a half-life of vWF Ag as 565 ± 35 minutes (mean ± SEM) [44], and vWF Ag kinetics after dDAVP infusions are not different in uremic patients and in controls [8]. High plasma vWF Ag levels could also be related to a hyperstimulation of vWF endothelial secretion pathways. VWF is an acute phase reactant protein. Thrombin, IL-1, gamma interferon, endotoxin, fibrin monomers, and the surface assembly of the complement C5b-9 can rapidly release vWF from cultured endothelial cells in a calcium dependent manner *in vitro* [45]. High levels of vWF antigen have been found in vasculitis of different origin [46] and in septicemia [47], conditions with damage to the vascular endothelium, and also in several diseases known to affect the vascular system such as diabetes [48], active glomerulonephritis and peripheral artery disease [49]. Brinkhous and coworkers have reported increased plasma levels of vWF in dogs after experimentally-induced endothelial damage [50]. These findings have led to the proposal that raised plasma levels of vWF may indicate endothelial damage. Theoretically, platelet activation could also have contributed to the elevated plasma vWF Ag. Activation of platelets is known to be the rule in patients on long-term hemodialysis [51], and platelet activation has already been proposed to elevate plasma vWF Ag [52]. However, in normal subjects, platelet origin vWF represents only 10 to 25% of the plasma pool [53]. Platelet levels of our patients were within normal values and there is no evidence that a non-extreme platelet activation or lysis can really produce a significantly elevated plasma vWF Ag.

A hyperfibrinolytic state is frequently found in uremia, which is associated with increased plasmin generation [16, 17]. T-PA is the main active plasminogen activator in euglobulin clot lysis and fibrin plate assays. Like vWF, t-PA is mainly synthesized in endothelial cells and may be released under the influence of different stimuli: exercise, venous occlusion, vasopressin analogs, thrombin itself [54, 55]. However, IL-1 and TNF-α are known to reduce t-PA synthesis by human umbilical vein endothelial cells *in vitro* [56]. The *in vivo* half-life of t-PA is short: 3.1 minutes for t-PA activity and 4.1 minutes for t-PA antigen. The liver removes most of the freely circulating t-PA, t-PA/PAI complexes being eliminated at a lower rate than free t-PA [57]. The rapid plasma clearance of t-PA involves receptor-mediated endoyctosis, and occurs by way of the heavy chain, most exclusively in the liver [58]. Our patients did not have the usual clinical and biological indicators of hepatocyte insufficiency. The kidneys did not significantly alter the concentration of t-PA Ag and t-PA activity of the blood [57] and high t-PA Ag plasma concentrations cannot be a direct consequence of renal insufficiency itself. High basal plasma t-PA Ag levels should rather be explained by a non-IL-1 dependent or by a TNF-α-dependent endothelial stimulation, by vascular endothelium damage, or perhaps by both mechanisms.

High predialysis soluble thrombomodulin levels were found in the patients' plasma. Thrombomodulin is a glycoprotein with a molecular weight of approximatively 105 kD that is present on the cell surface membrane of endothelium [59]. The increase in the plasma-free TM level is considered to be a marker of endothelial injury [33]. In patients undergoing hemodialysis, a positive correlation has previously been found between the TM predialysis value and the duration of dialysis, and TM was proposed as a marker of vascular disorders [20]. TM plasma levels are also elevated in patients with acute renal failure and creatinine, and TM levels are positively correlated [60]. Soluble TM is found in human urine [61]. Plasma TM is probably physiologically filtrated into urine through glomerular membrane, as the molecular weight of soluble TM in plasma were found to be 28 to 105 kD [62]. However, due to the broad range of predialysis TM values observed in our patients with chronic renal failure, part of the high TM concentrations in patients is probably due to endothelial injury.

Patients had higher predialysis u-PA Ag values than controls. u-PA is synthesized by monocytes, epithelial cells and vascular endothelial cells [63]. Data obtained from the pharmacokinetic studies of purified single-chain u-PA establish a turnover in blood with an initial t1/2 of about three minutes, and a hepatic clearance [64]. IL-1 and TNF-α are known to induce the production of urokinase-type plasminogen activator by human endothelial cells [65]. Moreover, Van Hinsbergh et al [66] have demonstrated *in vitro* that human endothelial cells from aorta and other adult arteries start secreting u-PA after one to four passages, parallel to the occurrence of enlarged endothelial cells; the release of u-PA antigen from human macrovascular endothelial cells is related to the exhaustion to proliferate as a normal monolayer. This suggested that the release of u-PA Ag by human macrovascular endothelium could be an indicator of cell senescence.

Desmopressin induced vWF Ag and t-PA Ag releases were lower in patients than in controls. These data are concordant with those obtained by Winter et al [6], who used a 15-minute venous occlusion test to point to an impairment of endothelium capacities. Recent data in animal models have shown a close link between the release of t-PA and that of vWF at the endothelial level [67]. In this way, desmopressin-induced t-PA Ag and vWF Ag releases were correlated in the control and the patient group, but this correlation was weaker in dialyzed uremic patients. This probably indicates a partial impairment of t-PA and vWF release coupling at the cellular level. In patients desmopressin sometimes induced an abnormal increase of albumin concentrations. Because of similar dDAVP-induced packed cell volume variations in patients and controls, this indicates albumin transfer from the extravascular compartment to the intravascular compartment, and probably denotes an impairment of the endothelial permeability to macromolecules.

Thus, patients on prolonged maintenance hemodialysis with no cardiovascular antecedent have biological cardiovascular risk markers: high predialysis plasma levels of fibrinogen, proconvertin and type 1 plasminogen activator inhibitor, low or high antithrombin III levels, and low albumin concentrations. They also have high plasma basal levels of endothelium-derived glycoproteins: t-PA, vWF and u-PA. This should be related to high cytokine plasma levels and should indicate a chronic endothelium activated state. However, there is a sheaf of arguments for a chronic endothelial damage: high soluble thrombomodulin concentrations, reduced desmopressin induced vWF Ag and t-PA Ag releases. IL-1 and TNF-α are probably not in themselves injurious to the endothelium, but cytokine-treated endothelial cells may be more susceptible to injury, especially in activated leukocyte-induced endothelial injury models, and may explain that *in vivo* endothelial activation and endothelial injury often co-exist [68, 69]. Transient survivable disruptions of arterial endothelial cell plasma membranes are far more commonplace than previously suspected, and most of the mitotic endothelial cell are also

wounded cells [70]. This may lead, in cases of associated uremic levels of cytokines and toxins, to severe endothelial damage and could be linked to the initiation of atherosclerosis. Moreover, the accumulation of dialysis treatments seems to be associated with a loss of desmopressin induced variations of vascular permeability and of t-PA/vWF release. This could argue for a direct effect of repeated dialysis runs on endothelial integrity, but the effects of uremia and the effects of dialysis treatment *per se* are impossible to separate.

Acknowledgments

We thank the nurses and the medical staff of the Haemodialysis Treatment Unit of the Department of Nephrology, CHU Nîmes, for their help in performing this study.

Reprint requests to Jean-Christophe Gris, M.D., Ph.D., Consultation et Laboratoire d'Hématologie et Immunologie, Centre Hospitalier Gaston Doumergue, 5, rue Hoche, BP 26, 30006 Nîmes cedex, France.

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